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Short Communication

Induction of chlorophyll mutants in *Zingiber officinale* Roscoe by Gamma Rays and EMS

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Abstract: The present investigation comprises the data on frequency percentage of chlorophyll mutations in the different doses of gamma rays coupled with EMS in ginger. Different mutation frequencies and width of mutation spectra were induced under the action of different concentrations of the two applied mutagens. The spectrums of chlorophyll mutations (albino, xantha, and chlorina) were observed and grouped. The overall mutation spectrum for ginger showed that xantha occurred with the highest frequency, followed by chlorina and albino. The mutagenic effectiveness decreased with the increase in dose of mutagen for both the mutagen indicating that negative relationship between effectiveness and dose of mutagen. EMS provided more number of chlorophyll mutants than gamma rays in this investigation.

Keywords: Ginger, Chlorophyll mutants, EMS, Gamma rays.

استحثاث الطفرات لمادة الكلورفيل فى نبات الزنجبيل *Zingiber officinale* Roscoe بواسطة اشعة جاما و EMS

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الملخص: يتضمن هذا البحث بيانات عن نسبة تواتر الطفرات لمادة للكلورفيل في جرعات مختلفة من أشعة جاما إلى جانب EMS لنبات الزنجبيل. وقد تم تبنى الطفرة التي تسببها ترددات مختلفة وأطياف بواسطة عدة تراكيز لاثنين من الطرق التطبيقية في هذه الدراسة. وكانت أطياف الطفرات عبارة عن alibino, xantha, and cholrina تم ملاحظتها وتجميعها. وقد بينت النتائج الكلية أن الطفرة لطيف xantha لنبات الزنجبيل حدثت وفقا لأعلي تردد يليها chlorine و albino وقد بينت النتائج أن اختلاف الجرعات من المادة المتطفرة ذو علاقة سلبية ببين الفعالية والجرعات المتطفرة وذلك بعد انخفاض فعالية المادة المتطفرة مع زيادة الجرعة. وقد بينت النتائج النهائية أن EMS قدمت عددا اكبر من الطفرات لمادة الكلورفيل أكثر من أشعة جاما في هذه الورقة العليمة.

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Introduction

Zingiber officinale Roscoe (Ginger) a member of the family Zingiberaceae is an important tropical horticultural plant, valued all over the world as a spice in culinary preparations and for its medicinal properties. Breeding of ginger is seriously handicapped by poor flowering and seed set. Most crop improvement programmes of this species are confined to evaluation and selection of naturally occurring variations. One of the most possibilities is the genetic marker system to find out the genetic variation (Arulbalachandran et al., 2009). The mutation breeding provides an alternative means of induction of variation. The induced mutations have been used to improve agronomic traits of many crops. Use of ionizing radiations, such as X-rays, gamma rays, neutrons and chemical mutagens for inducing genetic variation is well established (Mlcochova et al., 2004). The chlorophyll mutation frequency in mutation generation is the most dependable index for evaluating the genetic effects of mutagenic treatments (Kharkwal, 1998; Waghmare and Mehra, 2001). According to Von Wettstein et al., (1971) nuclear genes control the biogenesis of plastids. Chlorophyll mutants are used as tests for evaluation of genetic action of mutagenic factors (Gaul, 1964; Svetleva, 2004). According to Brunner (1995) selection of mutants usually begins in the second generation or later generation for the Vegetatively Propagated Plants (VVP) because it will be much easier to recognize mutants as they segregate out. Gamma irradiation as a mutagen can induce useful as well as harmful mutation in plants (Gupta, 1996; Micke and Donini, 1993). The aim of the work was to study the effect of Ethyl Methane Sulphonate (EMS) and Gamma rays on induction of different types of chlorophyll mutants and to determine their frequency in ginger.

Materials and Methods

The experiment was conducted at the Department of Botany, Shivaji University, Kolhapur. The rhizomes of the healthy ginger were exposed to gamma rays emitted from Cobalt-60 source with different level of doses (0.125, 0.250, 0.375, 0.500, 0.750 and 1.00 KR) at Bhaba Atomic Research Center, Mumbai, with dose rate 2.8 KR per minute. The same size of the rhizomes was administered for creating variability with chemical mutagen EMS. Treatments were carried out with chemical mutagen (EMS) at concentrations of 0.10, 0.15, 0.20 and 0.25% for four hour presoaking and 0.30, 0.40, 0.50 and 0.60 % for eight hour presoaking.

As a post treatment care the shoot tips were washed in running water for 45 minutes. Both gamma rays irradiated and EMS treated rhizome were grown along with control (Untreated seeds) by Randomized Block Design (RBD) with three replications at the breeding field. Before planting, bits should be treated with fungicide as a safeguard. Space were kept 25-45 cm between rows and 15-20cm between plants. Initial watering at an interval of 7 days was given to beds. Chlorophyll mutation observed when the seedlings were 50 days old and classified according to Gustafsson (1940) as follows.

Albino: they were characterized by total absence of either chlorophyll or caretenoids.

Xantha: these mutants were distinguished by their uniform yellow colour with total absence of chlorophyll.

Chlorina: these mutants had uniform yellow green colour. They emerged along the green seedlings and survive upto maturity.

Results and Discussions

Almost all the mutagenic treatments showed different degree of mutants with respective dose. In the present investigation, some of the chlorophyll mutants were observed in the different dose/concentrations of gamma rays and EMS they were chlorina, albino and xantha. Different chlorotic abnormalities

were scored in VM1 and VM2 (Figure 1) and results of the same are depicted in Table 1 and 2. The correlation of chlorotic mutants percentage in VM1 and VM2 shows good relation $R^2 = 0.801$ (Figure 2). The percentage of xantha mutants was highest in both the generation followed by chlorina while percentage of albino was lowest in both VM1 and VM2. The trend of the mutation percentage was not correlated with increase or decrease in dose of EMS 4 hrs (VM1) and EMS 8 hrs (VM1 and VM2) treatments. The percentage of chlorotic abnormalities per plant showed gradual decrease with increasing EMS 4 hrs treatments in VM1 and VM2.

Table 1. Percentage of chlorophyll mutants in VM1 generation of Ginger after mutagenic treatment.

Treatment	Concentration	Total mutation (%)	Individual mutants (%)		
			Albino	Xantha	Chlorina
Control	--	--	--	--	--
EMS 8 hrs	0.10%	19.04	4.76	9.52	4.76
	0.15%	20.83	8.33	8.33	4.16
	0.20%	18.18	4.54	13.63	--
	0.25%	15.00	5.00	5.00	5.00
EMS 4 hrs	0.30%	22.72	9.09	9.09	4.54
	0.40%	16.66	4.16	8.33	4.16
	0.50%	13.04	4.34	4.34	4.34
	0.60%	12.5	6.25	6.25	--
Gamma Rays (KR)	0.125	19.04	4.76	9.52	4.76
	0.250	19.04	4.76	9.52	4.76
	0.375	12.5	--	6.25	--
	0.500	14.28	--	14.28	--
	0.750	--	--	--	--
	1.000	--	--	--	--

Table 2. Percentage of chlorophyll mutants in VM2 generation of Ginger after mutagenic treatment.

Treatment	Concentration	Total mutation (%)	Individual mutants (%)		
			Albino	Xantha	Chlorina
Control	--	--	--	--	--
EMS 8 hrs	0.10%	14.28	4.76	4.76	4.76
	0.15%	16.66	4.16	8.33	4.16
	0.20%	13.63	4.54	9.09	--
	0.25%	10.00	5.00	5.00	--
EMS 4hrs	0.30%	16.66	4.16	8.33	--
	0.40%	12.50	4.16	4.16	4.16
	0.50%	08.69	4.34	4.34	--
	0.60%	06.25	6.25	--	--
Gamma Rays (KR)	0.125	14.28	4.76	4.76	4.76
	0.250	14.28	4.76	9.52	--
	0.375	06.25	--	6.25	--
	0.500	14.28	--	14.28	--
	0.750	--	--	--	--
	1.00	--	--	--	--

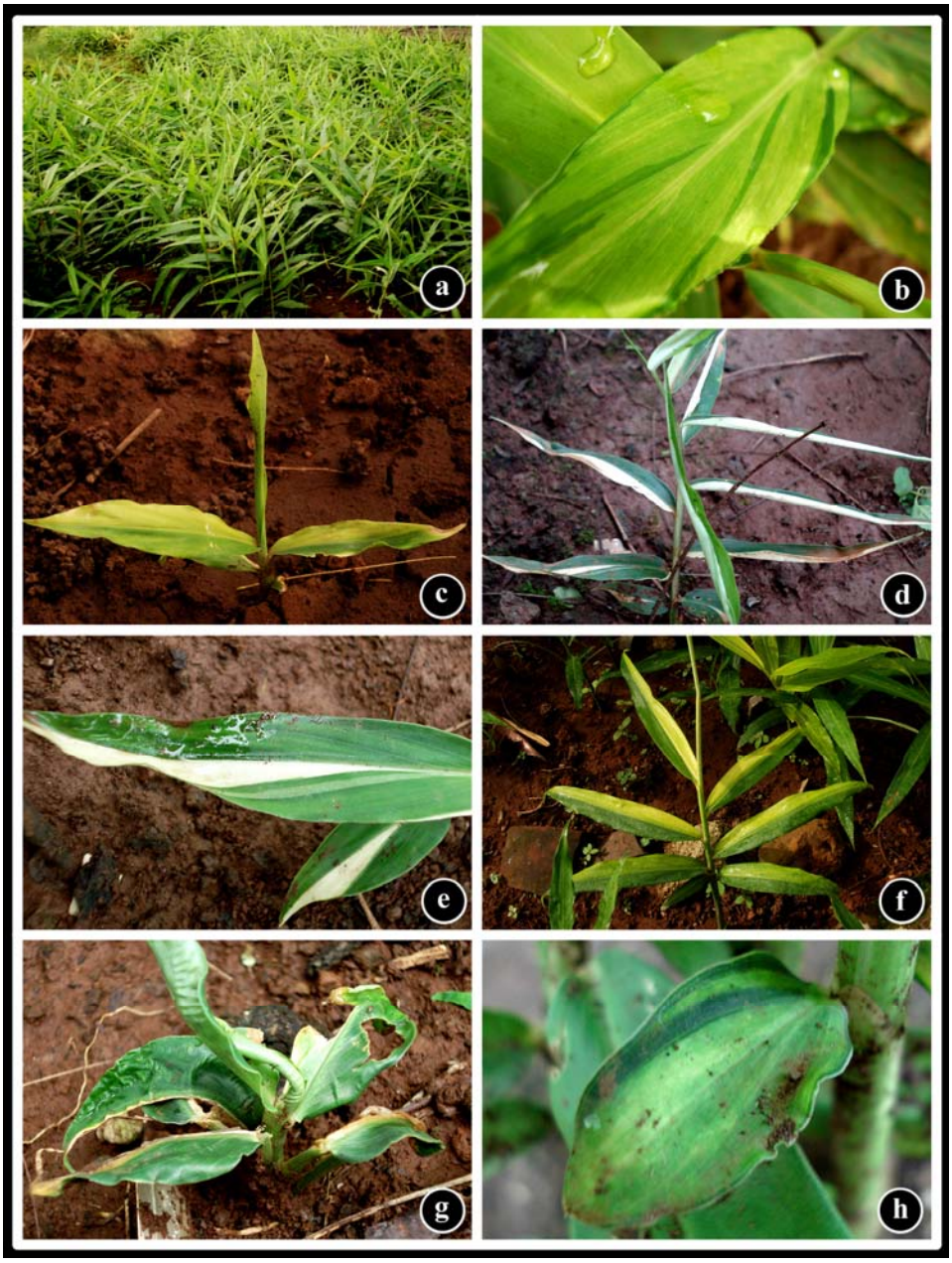

Figure 1. Gamma rays and EMS induced chlorophyll mutants in VM2 generation; a: Experimental plot; b: Chloro - Gamma rays 0.375 KR; c: Xantha - Gamma rays 0.250 KR; d: Albino - EMS 8 hrs. 0.15%; e: Albino - EMS 4 hrs. 0.40%; f: Xantha - EMS 8 hrs. 0.20%; g: Dwarf - Gamma rays 0.750 KR; h: Ovate leaf and Chloro - EMS 4 hrs. 0.60%.

In EMS 8 hrs treatment highest mutation percentage was recorded in 0.15% dose (16.66%) and lowest in 0.25% dose which was 10% (Table 2). In case of Gamma rays the percentage of mutants increased initially (0.250 KR) and then decreased with dose increased.

The phenomenon of albinism is rarely exhibited by plants which characteristic deficiency of chlorophyll and subsequent whitish-yellow colour of entire seedling (Hooda et al., 2004). These types of mutations were observed in mungbean (Singh and Singh, 1989) in chickpea (Kharkwal, 1999) and in grass pea (Waghmare and Mehra, 2000.) Chlorophyll development seems to be controlled by many genes located on

several chromosomes, which could be adjacent to centromere and proximal segment of chromosomes (Swaminathan, 1964). In present work EMS was to be superior to gamma rays in higher frequency percentage of chlorophyll mutations in VM2 generation, this is an agreement with work carried out by Swaminathan et al., (1970).

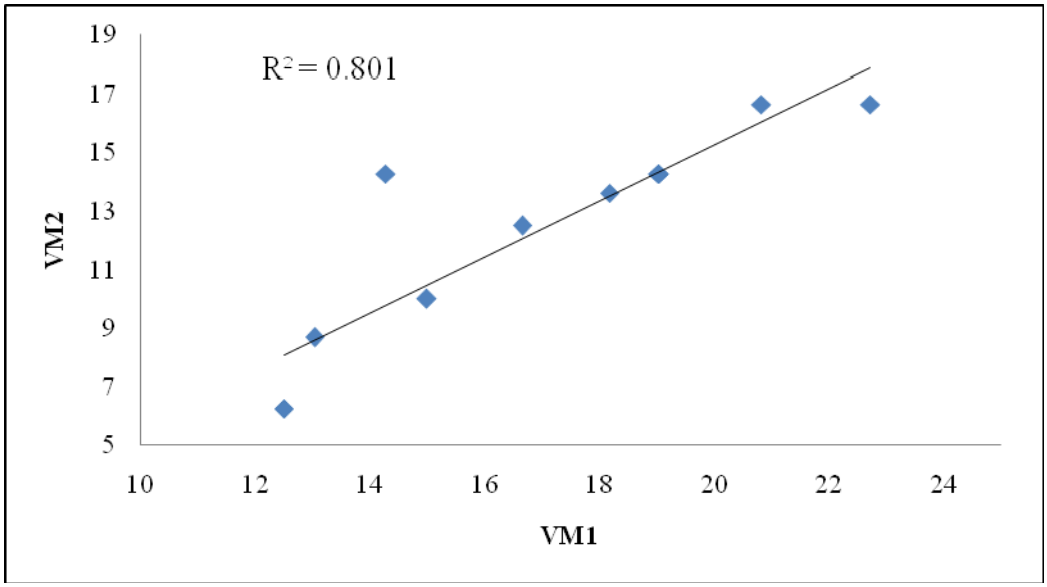

Figure 2. Correlation in mutation % between VM1 and VM2.

Conclusions

Present investigation revealed how chlorophyll gene response to mutagen gamma rays and EMS. Chlorophyll genes are reflected in the VM1 and subsequent generations in the form of different types of chlorophyll mutants which can be useful as marker in physiological and biochemical investigations.

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